1988

Serum Progesterone Levels as a Means to Study Reproductive Function in Female Lethal Yellow Mice (Ay/a; C57BL/6J)

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by

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A thesis submitted in partial fulfillment
of the requirements for the degree
Master of Science
Major in Biology
South Dakota State University
1988

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to Study Reproductive Function in
Female Lethal Yellow Mice (A^{Y}/a; C57BL/6J)

This thesis is approved as a creditable and indepedent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ACKNOWLEDGEMENTS

I am grateful to my graduate advisors, Dr. Peter J. Wilkin and Dr. Nels H. Granholm for their leadership and guidance during this course of study. I especially wish to thank Dr. Granholm whose wit and wisdom motivated me through this enduring task. Thanks for the patience and perseverance.

I am also grateful to Robert Japs, Jerry Dickens and Mary Ann Schoessler for their technical assistance and moral support in this study. I would like to extend my appreciation to Betty Petitjean-Wagner for her suprvision in completing the RIA's, Randy Vanbeak of SDSU Computer Center for his assistance in statistical evaluations, Cathy Haan for typing this manuscript, Dr. Charles R. McMullen for his financial support, and many others who provided much needed support and encouragement.

I am particularly grateful to my loving wife Nancy and three precious girls, Jennifer, Carrie and Britany for their unfailing support and financial sacrifice which made this thesis possible.

LITERATURE REVIEW

Since 1905 when Cuenot was first credited for bringing the (Ay/-) mouse to the attention of the scientific community, the yellow mouse has been used extensively in scientific research. The lethal yellow gene (Ay) of the agouti locus found on chromosome 2, linkage group 5 (Bray and York, 1979) in mice (Mus musculus) has many and varied effects. These effects include yellow coat color, increased fat deposition, increased body lengths, decreased tail lengths (Cizadlo, et al., 1975), increased cholesterol content in females, (Bartke and Wolff, 1966) increased susceptibility to lung tumors, lethality during embryonic development in homozygotes, and aberrant reproductive problems (Cizadlo, et al., 1975). All these varied phenotypic differences are due presumably to a single allele that exists in the yellow (Ay/a) which is different from the black (a/a) mouse.

This research concentrated on the reproductive problem associated with the Ay. Granholm and Brock (1981) reported that lethal yellow females never produced more than three litters when put into production at puberty. Ay/a females are as reproductively efficient as their a/a littermates at a young age. Ay/a females progressively become obese and reproductively senescent beyond the age of 120 days. The obese Ay/a females beyond 120 days exhibit lower uterine weights and depressed ovulation (Granholm et al., 1986). Upon treatment with exogenous pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), the reproductively

senescent Ay/a females' ovulatory rates were restored to near control levels (Granholm et al., 1986).

In mammalian reproduction the olfactory system provides for effective communication within some species. A pheromone in the urine of the male causes the medial basal region of the hypothalamus of the female mouse to secrete a neurohormone known as gonadotropin releasing hormone (GnRH) (Hadley, 1984). GnRH acts on the anterior pituitary gland stimulating the synthesis and release of two gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH along with prolactin, which is also secreted by the anterior pituitary gland, regulate the cellular and endocrine functions of the ovary.

The hypothalamus stimulates the production of prolactin by the pituitary differently than the gonadootropins. The hypothalamus produces dopamine, a catecholamine, which depresses the production of prolactin. When the dopamine is reduced, the prolactin production increases; thus this is known as prolactin inhibitory factor (PIF). Dopamine is a neurotransmitter that is found in several parts of the brain, especially in the midbrain, where it travels to receptors on the lactotrophs in the anterior pituitary which regulate prolactin synthesis and release. High plasma prolactin concentrations have been seen to increase dopamine turnover in the median eminence followed by depressed prolactin secretion (Johnson and Everitt, 1984).

As previously mentioned FSH, LH and prolactin regulate the cellular and endocrine functions of the ovary. LH stimulates the

stromal cells of the adult ovary to produce and release steroids, androgens, estrogens, and progestins, from the ovary. Growth and maturation of the follicles depends upon FSH; the number of mature Graafian follicles is positively correlated to the concentration of plasma FSH (Whittingham and Wood, 1983). The two major steroids, estrogen and progestagen, reflect the cyclicity of ovarian activity. Prior to ovulation estrogen is the dominant steroid and following ovulation progestagen is the dominant steroid. This cycle is called the estrous cycle. The need for this cyclicity is due to the two distinct functions of the female reproductive tract. It must act to transport gametes to the site of fertilization. This is the estrogen dominated cycle. Also it provides a site of implantation of the fertilized egg and supplies nutrients to the embryo. This is done in the progestagenic part of the cycle.

The ovary consists of stromal tissue containing the primordial germ cells known as oogonia. All oogonia have entered or will have entered their first meiotic division prior to or shortly after birth, thus becoming primary oocytes. The primary oocyte is surrounded by ovarian mesenchymal cells to form the primordial follicles. Follicular cells secrete a basement membrane, membrane propria, outside the cell. These primary follicles are arrested until puberty when they receive a signal to start development again. The primary follicle will rapidly increase in diameter due to the increase in diameter of primary oocytes. Also, the granulosa cells become several layers thick secreting a glycoprotein material that forms an acellular

layer, the zona pellucida between themselves and the oocyte. The stroma becomes condensed to the membrane propria to form these cells. Cells of the granulosa layer develop receptors for estrogen and FSH and the thecal cells develop LH receptors.

If the circulatory levels of FSH and LH do not coincide with the development of the FSH and LH receptors on the granulosa and thecal cells, they will undergo a process of atresia, forming scar tissue. FSH and LH causes the follicles to mature into Graafian follicles. The granulosa cells and theca cells proliferate to increase the size of the Graafian follicle, but the oocyte remains the same size. The results of the proliferation of thecal cells is two distinct layers, theca interna and theca externa. The primary oocyte, is surrounded by a dense mass of granulosa cells called the cumulus oophorus, is suspended in follicular fluid, connected by a thin stalk of cells to the peripheral granulosa cells.

During this growth of the follicle there is a steady increase in the synthesis of androgens and estrogens. Some of the granulosa cells start to secrete estrogen. LH stimulates theca interna cells to synthesize androgens from acetate and cholesterol. Granulosa cells, upon stimulus by FSH, convert androgens into estrogens. Estrogen can bind to receptors in the granulosa cells which stimulates proliferation and yet more estrogen receptors. Estrogen and FSH stimulate the appearance of LH-binding sites on the outer layer of granulosa cells. If a brief surge in the LH level does not coincide with the presence of the LH receptors on the outer granulosa layer,

these cells die. The LH surge causes terminal growth changes in the follicle resulting in ovulation. Also this LH surge marks the beginning of the follicle becoming a corpus luteum.

Shortly after the onset of the LH surge there is a rise in the output of follicular estrogen and androgen, which then declines to very low levels. The outer cells of the granulosa layer no longer convert androgen into estrogen but instead synthesize progesterone. LH stimulates the synthesis of progesterone via the newly acquired LH receptors.

After ovulation the follicle changes into a corpus luteum. This is known as luteinization. The corpus luteum secretes primarily progestagens. The corpus luteum is maintained by LH; also prolactin receptors are present. In nonpregnant females the corpus luteum will function for only 2 days in mice (Johnson and Everitt, 1984). Luteal regression or luteolysis involves the collapse of the corpus luteum thus terminating this process resulting in a decrease in the output of progestagens (Johnson and Everitt, 1984).

In the previous section the development of an individual follicle was described. In considering the relationship between a given follicle and the overall estrous cycle; the estrous cycle is the interval between successive ovulations. In the normal mouse this occurs every four to six days. The estrous cycle can be divided into four phases -- proestrus, estrus, metestrus, and diestrus. Follicular development occurs in proestrus and estrus resulting in ovulation, while metestrus and diestrus represents the luteal phase

(Whittingham and Wood, 1983). The cycle differs in length depending on whether the female has mated or not. If the female mouse has mated with a nonfertile male pseudopregnancy will occur in which the luteal phase will last 11-12 days. If the female fails to mate, the luteal phase lasts 2 to 3 days. This is due to the lack of stimulation from the penis on the cervix during coitus. The stimulated cervix sends messages to the CNS and activates the release of prolactin from the pituitary. There is some evidence that prolactin may participate in the regulation of steroidogenesis in the follicle enhancing the progesterone secretion in the luteal phase. Prolactin appears able to modulate the number of ovarian receptors for LH and so affect steroidogenesis indirectly. Thus prolactin is important in the maintenance of the corpus luteum (Johnson and Everitt, 1984).

As previously mentioned, the regulation of reproduction is controlled by the hypothalamus, producing GnRH which stimulates the pituitary to produce gonadotropins. The gonadotropin releasing hormone is also known as luteinizing hormone releasing hormone (LHRH) or luteinizing releasing factor (LRF). When GnRH was measured in the portal blood at one hour intervals, the levels were found to occur in a sequence of pulses known as circhoral rhythm (Johnson and Everitt, 1984). Thus the circhoral rhythm has also been found in blood levels of FSH and LH. Since the gonadotropins stimulate the production and release of ovarian hormones, the blood levels of the progesterone and estrogens are also circhoral.

A small increase in the level of estradiol, a form of estrogen,

in the circulating blood results in the decrease of GnRH. This is a negative feedback on the hypothalamus. If the estradiol concentration increases between 200% to 400% and remains high, the gonadotropin secretion is enhanced resulting in a surge of LH and FSH thus a positive feedback occurs (Johnson and Everitt, 1984).

With reference to feedback control of gonadotropin release, progesterone seems to operate just the opposite of estradiol. A higher plasma concentration of progesterone enhances the negative feedback of estradiol thus holding FSH and LH to low levels. High levels of progesterone also blocks the positive feedback effects of estradiol and inhibits the effects of LH secretions. Low plasma levels can facilitate the positive feedback effects of estradiol in inducing an LH/FSH surge.

A third ovarian hormone, inhibin has a negative feedback effect on FSH levels but does not effect LH output. Inhibin is produced by the granulosa cells of a Graafian follicle (Johnson & Everitt, 1984).

Several mouse mutants that have similar reproductive problems to those found in the Ay/- mouse. Since the decrease in reproductive efficiency coincides with the onset of obesity in the yellow Ay/a mice (Granholm et al, 1986), we will start with the genetically obese (ob/ob) mice. As reported by Swerdloff et al. (1978) the ob/ob mouse has multiple abnormalities of the endocrine system, including impaired growth, obesity, and temperature regulation thus appearing to have a general hypothalamic defect. To test the reproductive aberration, Swerdloff et al. (1978) used an acute LHRH response test in ob/ob

males. Following castration, they found that FSH and LH serum levels were elevated with the greatest increase in control lean males. When the castrated mice were treated with graded doses of testosterone, ob/ob mice were found to be very sensitive to the feedback inhibition of gonadotropins. These findings suggested that the hypothalamic-pituitary axis was not fully functional. Interpretation of why the bolus of LHRH markedly increase the serum LH levels 2-fold higher in the lean mouse versus the obese mouse included an increase in metabolism of LHRH, or a partial defect of pituitary gland, or even a chronic underestimation of the pituitary gland due to LHRH deficiency in ob/ob mice. To test the latter interpretation chronic treatment of LHRH was conducted. Chronic treatments failed to restore LH levels to normal; thus the ob/ob mice is thought to have a defect in the pituitary function.

Numerous studies have been done on mice homozygous for the autosomal recessive mutation diabetes (db) indicating that the hypothalamus may be the site of action of the db genetic locus (Bray and York, 1979). The db mice have physiological abnormalities that include hyperglycemia, hyperinsulinemia, obesity, thermoregulatory disturbances and sterilty of both sexes (Bray and York, 1979). Johnson and Sidman (1979) recorded measurements of pituitary and serum LH and FSH concentration in intact and gonadectomized males and females, serum LH response to GnRH, and results concerning ovarian function, uterine growth and vaginal cyclicity in db mutants as well as the GnRH content in the hypothalamus. Johnson and Sidman (1979)

found the reproductive tracts of intact mutant female mice appeared to be unstimulated, yet the response of the endometrium, the uteri and the vaginal cyclicity to estrogen was normal. The ovaries of the db females were found to have normal sensitivity to gonadotropin stimulation when exposed in situ to exogenous PMS. When the db ovaries were transplanted to control females the ovaries performed normally. Even though the ovaries were returned to normal sensitivity the gonadotropins, ovulation still did not take place due to a failure to produce the required endogenous surge of LH. Interpretation of this may be the infertility in db female mice is due to inadequate gonadotropin stimulation not from an unresponsive reproductive tract. Unresponsive feedback to the hypothalamus was indicated when serum concentration of FSH and LH were measured after gonadectomy. Neither the FSH or LH serum concentrations in mutants increased as much as it did in normal mice. Although the hypothalamus could be receiving normal pulses of steroids, perhaps hypothalamic tissue simply cannot release GnRH to the pituitary. Therefore, technically the feedback may be normal, but the hypothalamic response may be defective.

Johnson and Sidman also found an increase in the GnRH content of the hypothalamus of both intact and ovariectomized mutants suggesting a defect in the release of gonadotropins. This could be a defect in the CNS or the pituitary gland, but the finding of equivalent increases in serum LH for mutant and control mice after GnRH administration makes it seem more likely that the problem relates to inadequate GnRH release from the hypothalamus.

The fatty rat (fa/fa) has shown similar reproductive problems as the Ay animal. The female fa/fa rat has reduced fertility (Bray and York, 1979). Studies have indicated low circulating levels of estrogen. This was evident from the delayed vaginal opening, prolonged estrous cycles, and reduced uterine weights found in the fa/fa females (Bray and York, 1979). Low levels of serum estrogen have been observed with normal levels of serum gonadotropins suggesting that the hypothalamic-pituitary threshold to feedback inhibition of gonadotropins is defective (Bray and York, 1979).

Studies have also been conducted on mice homozygous for the hypogonadotrophic hypogonadal (hpg) mutation. The hpg mutant mice are deficient in GnRH thus these mice are characterized by underdeveloped reproductive organs, low serum and pituitary gonadotropin concentrations and a reduction in the concentration of pituitary GnRH receptors. When administered with exogenous GnRH, hpg mutants normalized and levels of serum and pituitary gonadotropins resulted (Charlton et al., 1983).

Young et al. (1985) transplanted normal fetal mouse hypothalamus to the third ventricle of the adult mutant hpg/hpg mice. The fetal hypothalamic tissue preoptic area transplants (POA) partly reversed the GnRH deficiency of hpg of both sexes only when the grafted tissue grafts formed functional anatomical connections with the median eminence of the third ventricle. Female hpg mutants bearing hypothalamic implanats for 30-256 days were shown to have normalized gonadotropin concentrations and increased GnRH receptors on

the pituitary up to 60% of the normal value. Serum FSH substantially increased to within the normal range, ovarian and uterine weights increased to over 74% of normal values, LH receptors increased the ovary and vaginal opening occurred about 23 days after POA grafting. Treated mutants also displayed prolonged periods of estrus along with a lack of cyclic ovarian activity suggesting a lack of appropriate neural control over GnRH secretion from the grafted tissue (Young et al., 1985).

Now that we have identified some of the mutants that have similar reproductive problems as the yellow (Ay) mouse, the next section will deal with what is known of the yellow (Ay)-induced infertility. Olfactory cues are important in the recognition of sexual partners and in the integration of sexual behavior. Male mice secrete a pheromone into their urine which causes gonadotropin release in females resulting in the synchronization of estrus on the 3rd night after pairing of females previously caged with only females (Whitten, 1973). The inability of the reproductively senescent heterozygous Ay female to breed may be due to pheromonal aberrations. Bartke and Wolff reported in 1966 that the male Ay/a did not produce this pheromone. When Bartke and Wolff's experiments were reproduced, no consistent

differences between Ay/a and non-yellow controls were found (Whitten, 1973). Granholm and Brock (1980) studied the effect of the Ay gene on mating preference in C57BL/6J Ay/a and a/a control littermates. No differences were revealed in either elapsed time to mating or in mate

selection in the first and second matings. As the onset of obesity occurred, reproductive inefficiencies became apparent.

Reproductive performance of Ay/a females greater than 120 days progressively decreases when compared to age-matched a/a littermates. Studies by Granholm et al. (1986) revealed that Ay/a females were 78.7% heavier than a/a controls and that the Ay/a females did undergo estrous cycling at a much reduced rate. Granholm and Brock (1981) mated Ay/a females to a/a males and Ay/a females to Ay/a males at puberty and found that third litters were rarely produced and fourth litters were never observed.

Evidence indicates that the reproductive tracts of the Ay/a females may be a poorer environment for developing embryos when compared to control uteri. The average mean litter size from the C57BL/6J Ay/a x a/a mating was lower than the mean from the reciprocal cross in which embryos developed in nonyellow uteri (Cizadlo et al., 1975). Bartke and Wolff (1966) reported a decrease in survival of yellow newborn.

The ovaries of the Ay/a have been found to weigh more than the age matched a/a ovaries but when compared to body weight the yellow ovaries weighed numerically less than the controls (Granholm et al., 1986). It has been postulated that the apparently heavier ovaries in the Ay/a females may simply have been an expression of increased and uniform adiposity in organs of Ay/a mice or that the ovarian steroids may be involved. Beamer et al. (1983) found that estrogen, in the absence of gonadotropins, acts on the ovary to promote increases in

ovarian weight. Also the uteri of Ay/a females have been found to weigh significantly less than a/a uteri (Granholm et al., 1986), once again suggesting ovarian steroid imbalance. Granholm et al. (1986) also showed that the Ay/a females had lower ovulatory activity than the a/a littermates. When the 120-day plus Ay/a females were treated with exogenous gonadotropins the ovarian activity was restored. Thus more supporting evidence of steroid deficiencies.

Ovarian steroid deficiencies may be caused by numerous reproductive problems, one being the ovary itself could be defective in the yellow females. Dickens and Granholm (1986) performed reciprocal ovary transplantation between control (a/a) and yellow ·/a) females to determine if the reproductive failures are intrinsic extrinsic to the ovary. Dickens and Granholm (1986) found that the llow ovary performs as well as the black ovary in a black mouse and ıe black ovary performs as poorly in the yellow mouse as the original ellow ovary. This indicates that there is no intrinsic problem with the ovary and adds more evidence to the hostile environment induced by the Ay gene.

The poorer (hostile) environments as described by Cizadlo et al. (1975) for embryos was indicated by their experiments on mating characteristics and embryonic losses in the yellow mouse. Cizadlo et al. (1975) showed that the average mean litter size of 4.2 ± 0.7 (mean ± standard error of the mean) from strain C57BL/6J Ay/a x a/a mating was significantly lower ($P < 0.05$) than the mean of 6.1 ± 0.1 from the reciprocal cross in which embryos developed in nonyellow uteri. In

the Ys/ChWf strain, litter size from yellow female by black male matings (5.4 ± 0.1) was significantly reduced ($P < 0.05$) over the reciprocal cross (6.5 ± 0.1); in addition, there was a deficiency of Ay/a progeny born to Ay/a females suggesting a specific deleterious uterine effect of genetically yellow uteri (Ay/a) on heterozygous yellow (Ay/a) embryos (Cizadlo et al., 1975).

To summarize our knowledge regarding reproductive problems of the Ay/- mouse, Ay does not appear to cause obvious lesions in pheromonal communications and neurosecretory competence of young Ay/a mice. As Ay/a females age and the progressive onset of obesity is correlated with the loss of fertility, conditions of the aged yellow mouse are consistent with steroid deficiencies. Granholm (personal communication) stated his interpretations of the effects of Ay as:

a) Ay/a pituitary glands are producing insufficient quantities of gonadotropins or they are producing normal levels of defective (biochemically altered) gonadotropins,
b) Control levels of normal gonadotropins are being produced and secreted, but their concentration at the ovary is low because they are being incorporated into excess fat tissue. There simply is not enough gonadotropin acting on follicular ovarian cells to promote ova maturation and ovulation,
c) Control levels of normal gonadotropins are being produced, secreted and presented to ovarian target cells. However, Ay is affecting in some way the abillity of these target cells to receive, internalize and respond to the normal signals, and/or

d) Combinations of a, b and c or all of them.

Granholm and Dickens (1986) conducted a study to determine if the reproductive failures in aging obese lethal yellow (Ay/a) females are due primarily to intrinsic defects within Ay/a ovaries or to defects, systemic in nature, which are extrinsic to Ay/a ovaries. The extrinsic defects could be the result of aberrant gonadotropins, hypothalamo-pituitary problems, ovarian steroid-hypothalamus feedback abnormalities, and/or others (Dickens and Granholm, 1986). Reciprocal ovary transplantations between control black (B) and lethal yellow (Y) females, and transplants within the genotypes indicated no consistent differences in either a/a or Ay/a ovary performance regardless of the host's genotype. The four types of grafts (B-B, Y-Y, B-Y and Y-B) have yielded mean litters per female of 3.3, 2.0, 2.8 and 3.3 along with mean progeny per litter of 4.8, 4.8, 4.6 and 4.5, respectively. This data suggest that the reproductive failures associated with the lethal yellow gene (Ay) are not due to intrinsic ovarian lesions but rather to primary Ay induced defects operating extrinsically to the ovary (Granholm and Dickens, 1986).

By measuring the serum levels of progesterone during the estrous cycle and pregnancy via radioimmunoassay (RIA), we may be able to substantiate whether the ovary is functioning normally. If the reproductively senescent Ay/a females have normal levels of progesterone, the Ay/a ovaries would be functionally normal with respect to progesterone synthesis and release. In contrast depressed progesterone levels would not allow us to differentiate between any of

the alternatives (a-d) listed previously. This experiment was conducted to test the hypothesis that the profiles of serum progesterone levels in reproductively obese Ay/a mice will be significantly different from those of age-matched control (a/a) littermates.

In mice the ovary has to be present throughout gestation, as it is the main source of progesterone (Pointis et al., 1941). Ovariectomy at any stage will result in termination of pregnancy, except for the last day or two of gestation. Progesterone RIA's have been conducted by several scientists on mice approximately 60-70 days of age. Murr et al. (1979), McCormack and Greenwold (1979), Virgo and Bellwald (1974), Pointis et al. (1941) and others have found that plasma progesterone rises shortly after copulation in a variety of mice strains including a four-way cross of C strain, A strain of Strong, Swiss mice, Albino Swiss mice, and Swiss-Vancouver mice. The plasma progesterone level reaches a plateau from day 3 to day 9, a slight drop on day 10, rises to a peak around day 15, followed by a continuous decline to the day of parturition. Apparently the pituitary regulates the production of progesterone until day 10 of gestation; then it is believed the placenta assumes the steroid-regulating role (Murr et al., 1974). A sharp rise of plasma progesterone concentration of days 11 and 12 may be correlated to the rapid growth of the corpus luteum (Pointis et al., 1981). It has also been shown that the placenta during the second half of gestation secretes progesterone (Pointis et al., 1981). Compared to the ovary,

the placenta adds a very low contribution to the overall concentration of progesterone (Pointis et al., 1981).

Prior to the cessation of estrus in most rodents, they undergo a period of irregular cyclicity. This period of irregularity in the C57BL/6J mice shows up as prolonged cycles which is common among aging rodents (Nelson et al., 1981; Flurkey et al., 1982). Following the cessation of estrus, there is a period of persistent vaginal cornification (PVC) (Nelson et al., 1981). With proestrus being designated as day 1, day 2 - estrus, day 3 - metestrus, and day 4 - diestrus in the 4-day cycle, it was found that the midday plasma progesterone showed no significant age difference in cycling mice. In older mice, the midday cycle elevation of progesterone on day 3 was about 25% lower in older mice (12 months), but was not quite significant. The plasma progesterone in intact, PVC mice was 50% lower than basal values of cycling mice in either age group. This evidence suggests that this hormonal imbalance of progesterone may contribute to the pathology and dysfunction of the reproductive system of aged female rodents.

By comparing data received on serum progesterone levels via RIA with data provided by Nelson et al. (1981) on progesterone levels of normal cycling mice and with data recorded by Murr et al. (1974), Pointis et al. (1981) and others on progesterone levels in pregnant mice we can analyze the effect of Ay/- on ovarian steroids in both cycling and pregnant Ay/- mice.

Objectives

Objectives of this study were: (1) to assess the status of progesterone via radioimmunoassay (RIA) in pregnant 120 day +, obese Ay/a mice and in 120-day + reproductively ineffective obese Ay/a mice and (2) to determine if the Ay lesion intrinsically or extrinsically induces ovarian dysfunction.

MATERIALS AND METHODS

The objective of the study was to determine the status of plasma progesterone concentrations by means of radioimmunoassay (RIA) in pregnant and infertile Ay/a mice and control (a/a) littermate females.

Progesterone levels were then compared to degree of obesity, growth of secondary reproductive organs, fertility and estrous cycling in Ay/a and age-matched control (a/a) mice. The following hypothesis was tested in this experiment. Profiles of serum progesterone in reproductively senescent obese Ay/a mice will be significantly different (presumably lower) than those of age-matched control (a/a) littermate females.

Inbred mice (strain C57BL/6J-Ay/a and a/a) were used for this study. All mice were derived from matings of stock mice from the laboratory of Dr. Nels Granholm at the South Dakota State University Animal and Range Science Small Animal Facility. Original stocks of C57BL/6J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Mice were housed in plastic shoebox-type cages with metal grid tops. Bedding of white pine shavings was changed weekly. Wayne Breeder Blox and water were provided ad libitum. Mice in the colony experienced a controlled light environment of 16 hours light and eight hours dark (on at 0600 and off at 2200). Records detailing date of mating, date of birth, and date of weaning were kept on cards attached to mating cages. All litters were weaned and sexed between 20 and 30 days of age. Age-matched and sexed weanlings were placed 12 to 15 per

cage in large aluminum cages and provided with Wayne Lab Blox and water ad libitum. Weaned females were kept in these cages until they were 120 days or older.

This actual experiment was carried out in three mating runs. For each run 50 proven a/a males were kept in individual cages numbered 1-50. Twenty-five earpunched 120-day-plus Ay/a females were housed in odd-numbered cages while twenty-five earpunched age-matched control (a/a) littermates were placed in the even numbered cages for the first and second runs. Each morning for six consecutive days, all females were checked for vaginal plugs indicating copulation during the previous night. Dates of copulatory plugs were recorded. Female mice showing plugs were divided into approximately equal groups and sacrificed on days 0 (day of mating), 2, 5, 10 and 15. As reported by Murr et al. (1973), plasma progesterone levels rise for the first 3 days, reach a plateau from day 3 to day 9, decline on day 10, and rise to a peak again on day 15. Also, 5 Ay/a females and 5 a/a females which failed to mate were randomly selected; vaginal smears were prepared prior to sacrifice in each of the first two runs. The remaining Ay/a and a/a females were placed in large aluminum cages until the third run.

In the third run 35 Ay/a females and 15 of the a/a females which had failed to mate on the first two runs were used. Females that mated were divided into groups of day 0, 2, 5, 10 and 15 days gestation and sacrificed on the given day. Randomly selected unmated female Ay/a and a/a mice were sacrificed following preparation

of vaginal smears. Ten Ay/a and ten a/a females which failed to mate were returned to stock cages to be used in other experiments. The following data were collected for each of the sacrificed females: (1) Age at sacrifice, (2) Body weight, (3) Tail length, (4) Estrous stage, (5) Weight of right and left ovarian fat pads, (6) Weight of right and left gastrocnemius muscle, (7) Weight of right and left ovary, (8) Weight of uterus, (9) Number of normal and degenerative ova flushed from reproductive tracts, (10) Number of embryos found in either early cleavage or blastocyst stages, (11) Gestation day, (12) Number of deciduae and their mean weight, (13) Visual score of the right ovary to assess number of follicles, (14) Number of ova "punched" from the right ovary and whether they were naked (without granulosa cells), contained granulosa cells, or were in liberated follicles; (15) Progesterone levels, and (16) Histological data of left ovaries, including numbers of preantral follicles, small antral, Graafian follicles, corpus lutea, atretic follicles and stromal characteristics (see Methods and Materials). These raw data are listed in Appendices I and II.

Each female used in the experiment was weighed to the nearest 0.1 g. Vaginal smears were obtained from all unmated females by inserting the flat end of a wooden toothpick into the vagina and scraping caudally along the dorsal vaginal wall using the method described by Whitten and Champlin (1978). The sample was then spread on a labeled glass slide. Each smear was viewed under a Nikon phase contrast microscope at 200x for estrous stage determination. Criteria

for staging estrous phases in the mouse were provided by Rugh (1968). These include:

Phases of Estrus in the Mouse (Rugh, 1968)

Stage	Description of Vaginal Smears
Proestrus	Mostly nucleated and some cornified epithelial cells, approximately equal number of leukocytes as a nucleated epithelium.
Estrus	Both nucleated and cornified epithelium (not clumped). Very few or no leukocytes.
Metestrus	Clumped cornified epithelial cells. Leukocyte numbers increase as the cycle approaches diestrus.
Diestrus	Some nucleated epithelium, mostly leukocytes and some mucous.

Between the hours of 0930-1130, all females were anesthetized and decapitated. Blood was collected in test tubes, stored in ice, and centrifuged in a CRU-5000 IEL centrifuge at 2600 RPM for 30 minutes. The serum was separated and stored at -20^{o}C until the progesterone assays (RIAs) were conducted. Following sacrifice, tail lengths were measured from the anus to the tip of the tail to the nearest mm. Each mouse was then dissected; the ovarian fat pads, ovaries, oviducts, the entire uterus, and gastrocnemius were removed and weighed to the nearest 0.1 mg on a Mettler analytical balance. All organ weights were conducted in the following manner. Upon removal, the organ was placed on labelled weigh paper and weighed. The organ was then removed and the weigh paper reweighed. The organ weight was computed as the difference of these two weights. Oviducts

and uteri were observed under the dissecting microscope. Mice with either no record of mating or from 1-5 days pregnant were observed for the presence of ova and embryos in reproductive tracts. Reproductive tracts were assessed on the basis of the following: (1) Absence of ova or embryos, (2) Number of ova or embryos present in the ampulla of oviducts, and (3) Number of ova present in regions other than ampulla. If no ova could be detected in the oviductal ampulla, the entire oviduct and uterine horn was flushed with approximately 0.1 ml of Ringer's solution via insertion of a truncated 30-gauge needle into the ampulla of the oviduct according to the procedure of Rafferty (1970). The presence of ova in the ampulla indicated that estrus and ovulation has occurred the previous evening. In those uteri in which deciduae could be observed, each decidua was counted, dissected free of the uterus, and weighed to the nearest 0.1 mg.

Upon removal of ovaries, the left ovary was placed in 10% formalin fixative until the ovary was processed for light microscopy by the South Dakota State University Veterinary Diagnostic Laboratory. Thirty left ovaries were used in the histological study. These ovaries were comprised of fourteen yellow (Ay/a) ovaries including 1 ovary from day 0 of gestation, two ovaries from days 5, 10 and 15 of gestation, and 2 ovaries each from Ay/a females judged to be in proestrus, metestrus and diestrus along with one ovary from an Ay/a female in estrus. Of the sixteen control (a/a) ovaries used, two ovaries each were from mice in gestation days 0, 2, 5, 10 and 15, and two ovaries each were from females found in proestrus, estrus and

metestrus. Five consecutive serial sections were taken at 1/3, 1/2 and 2/3 of the way through each ovary for a total of 15 sections per ovary. The state of folliculogenesis in the Ay/a ovary was compared to that of the age-matched a/a ovaries by scoring for primary follicles, secondary follicles, Graafian follicles, and corpora lutea along with an assessment of ovarian stroma.

Right ovaries were placed in Ringer's solution, visually observed under a dissecting microscope at 30x, and the number of follicles was scored as follows: (1) Few follicles (0 to 5), (2) Moderate (6 to 12), and (3) Very follicular (13+). Each ovary was divided in half and "pricked" apart with 25-guage hypodermic needles using the technique of Rafferty (1970) to liberate ova from ovarian follicles. Each half was pricked 100 times. Liberated ova suspended in Ringer's solution were counted and scored as naked ova (no granulosa cells attached), ova with granulosa cells, and ova within small follicles.

Progesterone radioimmunoassays (RIAs) were conducted under the supervision of Ms. Betty Petitjean-Wagner, Department of Animal and Range Sciences RIA technician, South Dakota State University. The antibody coated tube progesterone double extraction assay was provided by Diagnostic Products Corporated, 5700 West 96 Street, Los Angeles, California 90045. All blood samples were brought to room temperature prior to use. Buffered I^{125} progesterone was used as the isotope. One minute counts were taken in Picker Compac 120 gamma counter.

Numerical differences and statistical differences between age-

matched Ay/a and control a/a mice were analyzed under the direction of Randy Vanbeak, Computer Center, South Dakota State University, using chi-square for continuous analysis of variance for discontinuous data.

Ay/a mice and control (a/a) animals were broken down into two study groups. Those between 120-180 days of age and those greater than 180 days at sacrifice. In Tables 1-7 statistical comparisons were conducted between young yellow and young black mice of 120-180 days of age and between old yellow and old black mice having ages greater than 180 days. Occasionally, all mice were pooled into either yellow or black genotypes and statistically compared. Significance was established at the $P < 0.05$ and $P < 0.01$ levels of probability.

All the raw data from this experiement are compiled in Appendixes IA, IB, IIA, and IIB.

RESULTS

General Reproductive Parameters

The same number of yellow and black females were used to mate with proven black males. Yellow and black females were age-matched and divided into two groups for comparison — those between the age of 120 and 180 days and those greater than 180 days of age (Table 1). The mean age of the yellow mice in the group between 120 days and 180 days of age at sacrifice was 137.4 ± 3.1, and the mean age of the black females in the same group was 139.7 ± 1.9 days at sacrifice. Yellow females greater than 180 days of age had a mean age of 246.5 ± 5.3 while blacks were 238.7 ± 5.6 days of age at sacrifice (Table 1).

Table 1 also shows a comparison of mean body weights in grams. Yellow females less than 180 days of age were heavier ($P < 0.01$) than age-matched black animals. The yellow animals greater than 180 days of age had mean body weights that appeared heavier than the black age-matched animals, mean weights of 40.9 ± 1.1 to 25.0 ± 1.2 even though not statistically significant at the $P < 0.05$ level. The overall weights of the four groups showed that the yellow animals had greater body weight ($P < 0.01$) than black mice.

We did not observe differences in tail lengths between the age-matched yellow and black female mice. Cizadlo et al. (1975) reported decreased tail lengths as one of the varied effects of the lethal yellow gene. Our observation indicates that the tail lengths do not increase between the two age groups of yellow mice (87.1 ± 0.6 mm to 87.2 ± 0.6 mm) whereas growth is shown in the age-matched black

Table 1. Comparisons of reproductive parameters in lethal yellow (Ay/a) and control (a/a) mice.

	Lethal yellow (Ay/a)		Black	
	< 180 days[1]	> 180 days	< 180 days	> 180 days
Number of females	27	23	29	21
Mean age (days)	137.4±3.1[2]	246.5±5.3	139.7±1.9	238.7±5.6
Mean body weight (gms)	30.5±1.3[a6] (16)[3]	40.9±1.1 (21)	24.6±0.8[b] (24)	25.0±1.2 (16)
Mean tail length (mm)	87.1±0.6 (16)	87.2±0.6 (21)	85.1±0.7 (22)	87.9±0.6 (16)
Mean wt of RO (mg)	5.33±0.7 (16)	5.2±0.6 (20)	4.7±0.5 (24)	4.8±0.7 (16)
Mean wt of LO (mg)	3.9±0.4 (16)	4.3±0.5 (20)	3.8±0.3 (24)	3.4±0.4 (16)
Mean % body weight RO (%)[4]	$0.02\pm2\times10^{-3}$ (16)	$0.1\pm1\times10^{-3}$ (21)	$0.2\pm1\times10^{-3}$ (24)	$0.2\pm2\times10^{-3}$ (16)
Mean % body weight LO (%)[4]	$0.01\pm1\times10^{-3}$ (16)	$0.01\pm1\times10^{-3}$ (20)	$0.02\pm1\times10^{-3}$ (24)	$0.01\pm1\times10^{-3}$ (16)
Mean wt nonpregnant uteri (mg)	62.7±5.4 (5)	51.2±4.5 (12)	64.9±5.1 (10)	62.8±3.8 (11)
Mean progesterone conc (ng/ml)[5]	13.9±3.3[ac] (16)	10.2±2.6 (21)	6.2±1.4[b] (24)	9.9±3.1[c] (16)

[1]There were two populations of mice based on age. One group was less than 180 days of age (< 180 days), the other greater than 180 days of age (> 180 days).
[2]Mean ± SEM.
[3]Number of females assessed.
[4]Weight of ovary divided by weight of the female x 100.
[5]Includes all females in the study, both pregnant and nonpregnant.
[6]Means in the same row sharing different superscripts are significantly different ($P < 0.05$).

animals.

The mean ovarian weights appeared to differ between the two genotypes with the yellow ovaries weighing more than the black controls (Table 1). But our comparisons did not show significant differences between the two genotypes. Our data do agree with the findings of Granholm et al. (1986) with Ay/a ovaries weighing more than a/a controls. When the right and left ovarian weights were compared to the body weight of the animals, there were no differences between the yellow and black mice.

The mean weight of nonpregnant uteri in the yellow animal was consistently smaller than the control black animal. Young animals, less than 180 days of age, did not vary in size, with the mean of the yellow nonpregnant uteri being 62.7 $\pm$ 5.4 mg and the control black nonpregnant uteri weighing 64.9 $\pm$ 5.1 mg. Older animals greater than 180 days of age the uteri of both genotypes decreased in weight, most noticeably in the yellow nonprgnant uteri 51.2 $\pm$ 4.5 mg, whereas, the black control nonpregnant uteri weighed 62.8 $\pm$ 3.8 mg. The largest difference between the two age-matched genotypes occurred in the older group of animals.

Table 1 shows the mean progesterone levels measured in ng/ml of the age-matched yellow and black mice. The yellow (Ay/a) females less than 180 days of age had the highest progesterone level (13.9 $\pm$ 3.3). This was significantly higher ($P < 0.01$) than their age-matched control (a/a) females (6.2 $\pm$ 1.4 mg/ml). In mice greater than 180 days of age, plasma progesterone levels were not significantly

different (10.2 ± 2.6 ng/ml in the yellow female animal and 9.9 ± 3.1 mg/l in the black female animal).

The control (a/a) females showed a significant ($P < 0.01$) increase in blood progesterone levels as they aged. As the Ay/a females aged, the plasma progesterone levels declined. In comparing genotypes, the yellow animals had a mathematically higher progesterone level (11.8 ± 2.0 ng/ml) than the black groups (7.7 ± 1.5 ng/ml). These mean total figures were not significantly different.

Relationships between body composition of fat and lean tissue are displayed in Table 2. The same animals used in Table 1 were used in this study. Upon comparing the ovarian fat pads and the gastrocnemius of the age-matched Ay/a female mice and the control (a/a) females, we expected to find an increase in fat deposition. As expected, there was a significant difference ($P < 0.01$) in both the right and left ovarian fat pads of the age-matched genotypes. The yellow females had the larger ovarian fat pads in both age-matched study groups. The percentage of body weight the ovarian fat pads comprised was also significantly higher ($P < 0.01$) in the yellow animals compared to the black animals supporting the findings of others that the yellow mouse has an increased fat deposition with age. The lean tissue represented by the right and left gastrocnemius of age-matched yellow and control black female mice showed no significant differences. Yellow mice greater than 180 days of age had the largest gastrocnemius by weight; however, when the percentage of body weight the gastrocnemius comprised was compared, control black mice had the

Table 2. Comparisons of fat and lean tissue in lethal yellow (Ay/a) and control (a/a) mice.

	Lethal yellow (Ay/a)		Black (a/a)	
	< 180 days[2]	> 180 days	< 180 days	> 180 days
Number of females	16	21	24	16
Mean wt, RO fat pad (mg)	$25.5^{a}\pm5.4$[5]	48.1 ± 4.6	$8.0^{b}\pm1.2$	$12.1^{ac}\pm3.5$
Mean wt, LO fat pad (mg)	$16.8^{a}\pm3.1$	$56.7^{c}\pm6.6$	$6.4^{b}\pm0.9$	$7.9^{bd}\pm2.0$
Mean wt, right gastrocnemius (mg)	83.8 ± 6.5	85.9 ± 7.0	79.2 ± 5.8	81.4 ± 6.3
Mean wt, left gastrocnemius (mg)	84.7 ± 5.7	91.6 ± 5.8	88.8 ± 4.3	90.2 ± 4.4
Mean percent body wt of RO fat pad (%)[3]	$0.08^{a}\pm0.01$	$0.12^{b}\pm0.01$	0.03 ± 0.01	0.05 ± 0.01
Mean percent body wt of LO fat pad (%)[3]	$0.05^{a}\pm0.01$	$0.13^{c}\pm0.01$	$0.03^{b}\pm0.01$	0.03 ± 0.01
Mean percent body wt of right gastrocnemius (%)[4]	0.29 ± 0.03	0.21 ± 0.02	0.33 ± 0.03	0.33 ± 0.02
Mean percent body wt or left gastrocnemius (%)[4]	0.29 ± 0.02	0.22 ± 0.01	0.36 ± 0.02	0.36 ± 0.02

[1]Ovarian fat pad and gastrocnemius muscle tissue were used to estimate fat and lean body composition, respectively.
[2]There were two populations of mice based on age. One group was less than 180 days of age (< 180 days), the other greater than 180 days of age (> 180 days).
[3]Weight of ovary divided by the weight of female x 100.
[4]Weight of gastrocnemius divided by weight of female x 100.
[5]Mean $\pm$ SEM. Means in the same row having different superscripts are significantly different ($P < 0.05$).

larger percentage of lean tissue.

Reproductive tracts of the age-matched yellow and control black mice (Table 3) were examined for the presence of ova, embryos and deciduae. Ova flushed from the tracts were scored either as normal or abnormal. Embryos were scored as early cleavage stage (day 0 to day 2 of gestation) and blastocyst (day 3 to day 5 of gestation). Deciduae were counted in each pregnant mouse and mean decidual weights were taken for the gestation days of day 5 and day 10 for comparative purposes between yellow and black females to determine if the uterine environment within the two genotypes influences their development.

The mean number of normal ova recovered from reproductive tracts of yellow females less than 180 days of age (3.4 $\pm$ 1.7) are similar to those of the age-matched black mice (4.1 $\pm$ 0.9). Ova recovered from the yellow females greater than 180 days of age (2.3 $\pm$ 0.9) were numerically (2.6 $\pm$ 0.8 and 4.6 $\pm$ 0.7 for Ay/a and a/a, respectively), but not significantly ($P < 0.05$) different. The mean normal ova from yellow females were lower (2.6 $\pm$ 0.8) than the control black females (4.6 $\pm$ 0.7). As expected the older yellow females shed fewer ova (2.3 $\pm$ 0.9) than the younger yellow females (3.4 $\pm$ 1.7).

The potential of ovaries to produce and liberate ova was tested by mechanical means. We adopted a method described by Rafferty (1970) in which excised ovaries in vitro are "pricked" by 20 gauge needles to liberate ovarian oocytes (see Methods and Materials). Liberated oocytes were pooled and counted to provide an index of ovarian activity. We used three scoring criteria for the liberated oocytes:

Table 3. Effects of lethal yellow gene (Ay) on ovarian and uterine parameters.

	Lethal yellow (Ay/a)		Black	
	< 180 days[2]	> 180 days	< 180 days	> 180 days
I. Ova and embryo recovery[a]				
Mean normal ova	3.4±1.7(7)[8]	2.3±0.9(15)	4.1±0.9(15)	5.3±1.0(12)
Mean abnormal ova[3]	0.3±0.3(7)	0.7±0.4(15)	1.2±0.6(15)	0.4±0.3(12)
Mean cleavage stage embryos[4]	0.0 (0)	2.5±0.4(2)	3.0±1.6(5)	0.0 (0)
Mean blastocyst embryos	2.0±1.4(2)	0.0 (0)	0.0 (0)	0.0 (0)
II. Uterine parameters				
Mean deciduae[5]	7.5±1.2(8)	8.6±0.9(5)	8.3±0.5(9)	8.3±0.6(4)
Mean wt of day 5 decid (mg)	2.2±0.0(1)	0.0 (0)	5.6±1.3(2)	3.8±0.4(2)
Mean wt of day 10 decid (mg)	55.2±5.1(4)	50.0±1.2(2)	53.4±0.7(2)	43.6±0.0(1)
III. Ovarian follicles-visual score[6]				
No. of ovaries assessed	16	21	24	16
0-5 follicles (%)	0	0	0	0
6-12 fofllicles (%)	0	0	4.0	19
13+ follicles (%)	100.0	100.0	96.0	81.0
IV. Mechanically liberated ova[7]				
No. of ovaries assesses	16	21	24	16
Mean naked ova	3.7±0.8	3.5±0.4	4.0±0.4	2.9±0.6
Mean ova with granulosa cells	8.0±1.0	6.9±0.8	6.4±0.8	6.9±1.4
Mean follicles containing ova	6.9±0.8	4.0±0.7	5.6[a]±0.7[9]	3.6[b]±0.5
Mean total liberated ova	18.6[a]±2.0	14.4[b]±1.0	16.0±1.5	13.4±1.7

Table 3. (continued)

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[1]There were two populations of mice based on age. One group was less than 180 days of age (< 180 days), the other greater than 180 days of age (> 180 days).
[2]Entire reproductive tracts were flushed with 0.85% NaCl to expell ova and embryos. Please see Methods for details.
[3]Abnormal ova were either granular or vesiculate.
[4]Includes 2-, 4-, and 8-cell stages plus morulae.
[5]Deciduae are the uterine swellings which occur as a result of the implantation process.
[6]Ovaries were scored for follicles by counting discrete surface swellings under a dissecting microscope.
[7]Ovaries were "punched" with 20-gauge needles to liberate ova and small ovarian follicles. Please see Methods for details.
[8]Mean $\pm$ SEM (n number).
[9]Means in same row with different superscripts are significantly different ($P < 0.05$).

(1) mean number of naked ova (without any granulosa cells attached); (2) mean number of ova with granulosa cells, and (3) mean number of liberated follicles with ova present (Table 3).

There were no differences in the ovarian activity of the age-matched yellow and black female animals. When the two age groups within a genotype were compared, the yellow mice greater than 180 days of age produced significantly ($P < 0.05$) less total liberated ova (14.4 ± 1.0) compared to the yellow female less than 180 days of age (18.6 ± 2.0). Black females greater than 180 days of age produced significantly less ($P < 0.05$) liberated follicles with ova (3.6 ± 0.5) when compared to the black females less than 180 ays of age (5.6 ± 0.7). The ovaries of all four groups appeared to be functional as judged by number of liberated ova.

Data from histological comparison of folliculogenesis in yellow and black female mice are summarized in Table 4. To observe histological differences between genotypes, ovaries of 14 yellow mice and 16 black mice were scored on the following criteria: (1) mean number of preantral follicles present (those with zona pellucida and thin layer of granulosa cells and membrane propria present), (2) mean number of antral follicles (those with zona pellucida, granulosa cells, membrane propria and thecal cells present), (3) mean number of Graafian follicles (follicles with follicular antrumand cumulos oophorus present), (4) mean number of corpora lutea (collapsed follicles characterized by absence of membrana propria between granulosa cells and thecal cells, vascularization of granulosa cells

Table 4. Histological comparison of folliculogenesis in lethal yellow (Ay/a) and control (a/a) mice.

	Lethal yellow (Ay/a)		Black	
	< 180 days[2]	> 180 days	< 180 days	> 180 days
Number of ovaries examined	9	5	8	8
Mean preantral follicles	6.3±0.8[2]	8.8±1.5	5.5±1.0	6.0±0.9
Mean antral follicles	2.8±0.5	2.2±0.4	2.8±0.6	2.2±0.4
Mean Graafian follicles	3.1^{bc}±0.5[3]	3.4^{a}±0.2	2.3±0.4	2.9^{b}±0.5
Mean corpora lutea	3.8±0.5	4.0±0.4	3.4±0.6	3.5±0.5
Mean atretic follicles	3.9±0.5	3.5±0.6	4.6±0.5	2.3±0.5
Stromal character[4] (%)				
Less than 50%	55.6(5)	60.0(3)	87.5(7)	62.5(5)
Equal to 50%	0	20.0(1)	0	0
Greater than 50%	44.4(4)	20.0(1)	12.5(1)	37.5(3)
Total ovarian follicles	19.1±2.0	21.9±1.9	18.5±1.8	16.9±2.2

[1]There were two populations of mice based on age. One group was less than 180 days of age (< 180 days), the other greater than 180 days of age (>.180 days).
[2]Mean ± SEM (n number).
[3]Means in same row with different superscripts are significantly different ($P < 0.05$).
[4]Stroma refers to supporting ovarian tissue not directly involved in folliculogenesis.

and yellowish to orange color), (5) the mean number of atretic follicles (degenerated follicles that appear as scar tissue), and (6) stroma character which was classified as less than 50% of total volume of cross section, approximately 50%, or greater than 50% volume of the total cross section of the ovary.

Of the fourteen yellow mice scored, five were greater than 180 days of age, four in various stages of the estrous cycle and one pregnant (day 15 of gestation). The other nine were less than 180 days of age, three mice were in various stages of the estrous cycle; of those remaining, one was in day seven of gestation, two in day five of gestation, two in day 10 of gestation, and one in day 15 of gestation. Thus, the total left ovaries of the yellow Ay/a mouse examined were two of each stage of estrous with the exception of estrous stage which we did not find in those animals greater than 180 days and two of each of the gestation groups (0, 2, 5, 10 and 15 days of pregnancy). Again we have some exceptions — we have only one day 0 and no day 2 yellow animals. Coincidentally, all the yellow animals in our day 2 group had false plugs.

Of the sixteen black animals, eight were greater than 180 days of age and eight were less than 180 days of age. Of the control mice greater than 180 days, all of the stages of the estrous cycle were represented. Day 0, day 5, day 10 of gestation were also represented. Those black animals less than 180 days of age were in proestrus and estrus stages of the estrous cycle. Days 0, 2, 5, 10 and 15 of gestation were also represented in control groups.

Data on folliculogenesis coincided with data on ovarian activity between age-matched genotypes. Numerically, ovaries of yellow and black mice were similar for the total population of animals measured in the categories of the mean number of control follicles. The largest numerical difference in total population comparison between the genotypes was found in the preantral follicles. The yellow (Ay/a) female mice have a mean number of 7.2 ± 0.4 preantral follicles and the black (a/a) female mice have a mean number of 5.8 ± 0.6.

The only significant differences in Table 4 were in the mean number of Graafian follicles. Yellow mice greater than 180 days of age were significantly ($P < 0.05$) higher (3.4 ± 0.2) than the age-matched black females (2.9 ± 0.5). Yellow mice greater than 180 days of age had significantly higher ($P < 0.05$) mean Graafian follicles (3.4 ± 0.2) than the yellow mice less than 180 days (3.1 ± 0.5).

In comparing stroma character, a higher percentage of yellow animals (35.7%) had greater than 50% of their total volume comprised of stroma than the black animals (25.0%). Those small differences document that Ay/a ovaries retain an amount of active ovarian tissue comparable to that of controls (a/a).

When mean progesterone concentrations were compared with the estrous stages between the two genotypes (Table 5), no significant differences were found. However, even with the small sample, several patterns seemed to develop. The progesterone concentration in the yellow mice seemed to decrease in the metestrus and diestrus stage as

Table 5. Serum progesterone concentration (ng/ml) and estrous stage comparisons in nonpregnant lethal yellow (Ay/a) and control (a/a) mice.

Estrous stage[2]	Lethal yellow (Ay/a)		Black	
	< 180 days[1]	> 180 days	< 180 days	> 180 days
Proestrus	(0)	3.6±2.3(3)[3]	2.0±0.3(2)	2.3±0.7(2)
Estrus	1.2±0.0(1)	2.1±0.0(1)	0.7±0.1(3)	1.6±0.6(3)
Metestrus	23.3±0.0(1)	3.5±2.6(2)	(0)	2.0±0.6(2)
Diestrus	16.0±13.0(3)	8.6±4.5(7)	2.4±0.0(1)	17.8±2.9(2)

[1]There were two populations of mice based on age. One group was less than 180 days of age (< 180 days), the other greater than 180 days of age (> 180 days).

[2]Estrous stages were determined by vaginal smears. Please see Methods.

[3]Mean ± SEM (n number).

they aged. In contrast, the black animals seemed to increase in progesterone concentration as they aged. Yellow mice greater than 180 days of age had progesterone concentrations numerically higher in all estrous stages than the age-matched black mice with the exception of diestrus. This also held true when all mice were compared. Yellow mice had numerically higher mean progesterone concentration than black mice with the exception fo those in diestrus.

Similar results were found in the comparison of the mean progesterone concentration and mating status of yellow and black mice (Table 6). When total mice were compared between the two genotypes, yellow mice had a numerically higher progesterone concentration. Yellow mice were significantly higher ($P < 0.01$) than the control mice that did not mate (no vaginal plugs and nonpregnant). Yellow mice less than 180 days of age had numerically higher progesterone concentrations in all mating parameters than the age-matched black animals.

Yellow mice less than 180 days of age with false plugs (vaginal plugs but not pregnant) had a mean progesterone concentration (13.4 ± 13.2) significantly higher ($P < 0.01$) than the age-matched control black mice (1.3 ± 0.4). Yellow mice less than 180 days of age that did not mate (no vaginal plugs and nonpregnant) showed significantly higher ($P < 0.01$) plasma progesterone levels than the age-matched control animals (7.7 ± 5.3 vs. 1.4 ± 0.3, respectively); yellow mice greater than 180 days of age also had significantly higher ($P < 0.01$) progesterone concentrations (4.3 ± 1.8) in the no vaginal plug and

Table 6. Serum progesterone concentration (ng/ml) and mating status comparisons in lethal yellow (Ay/a) and control (a/a) mice.

Mating status[2]	Lethal yellow (Ay/a)		Black	
	< 180 days[1]	> 180 days	< 180 days	> 180 days
Vaginal plugs and pregnant	13.7±3.7(9)	13.6±6.3(4)[3]	10.0±2.1(13)	24.1±9.9(3)
Vaginal plugs but not pregnant	13.4^{a}±13.2(2)[4]	8.0±7.5(2)	1.3^{b}±0.4(4)	9.1±6.7(4)
Nonplugged and nonpregnant	7.7^{abc}±5.3(4)	4.3^{a}±1.8(12)	1.4^{abd}±0.3(6)	1.9^{b}±0.3(7)
Nonplugged but pregnant	41.9±0.0(1)	30.9±1.8(3)	4.6±0.0(1)	17.8±2.9(2)

[1]There were two populations of mice based on age. One group was less than 180 days of age (< 180 days), the other greater than 180 days of age (> 180 days).

[2]Upon mating in mice, a copulatory plug forms in the vagina of the female. Such mice are termed "plugged". See Methods for details.

[3]Mean ± SEM (n number).

[4]Means in the same row with different superscripts are significantly different ($P < 0.05$).

nonpregnant category than the age-matched black animals (1.9 ± 0.3).

Progesterone concentrations in yellow mice seemed to decrease with age in all mating status categories. In contrast, black mice increase in serum progesterone concentrations with age in all of the four mating status groups.

Table 7 shows the comparisons of mean progesterone concentration at specific days of gestation. Day 0, day 2, day 5, day 10i and day 15 of gestation were used because they represent benchmarks described by Murr et al. (1974) in their study. Please see Materials and Methods for rationale in selecting these particular days of gestation.

Black (a/a) mice exhibited numerically higher progesterone concentrations than yellow mice in both age groups for every gestation day data was available. Black mice younger than 180 days of age in day 0 of gestation had a significantly ($P < 0.01$) higher progesterone concentration (7.9 ± 0.0) than the age-matched yellow females (0.9 ± 0.3). Day 10 of gestation black mice younger than 180 days of age also had significantly ($P < 0.05$) higher progesterone concentrations (42.0 ± 0.0) than the age-matched yellow (10.8 ± 0.8).

Progesterone levels increased as gestation progressed through day 15 in each mouse subpopulation with the exception of black mice less than 180 days of age; between day 10 and day 15, progesterone levels dropped by approximately 50% (Table 7). A drop of 21% was observed between the day 10 and day 15 of gestation in yellow mice greater than 180 days of age.

Table 7. Serum progesterone concentration (ng/ml) at specific days of gestation in lethal yellow (Ay/a) and control (a/a) mice.

Day of Gestation	Lethal yellow (Ay/a)		Black	
	< 180 days[1]	> 180 days	< 180 days	> 180 days
Day 0	$0.9^{a}\pm0.3(3)$[2]	----	$7.9^{b}\pm0.0(1)$	----
Day 2	----	----	$11.1\pm3.0(2)$	----
Day 5	$7.8\pm4.9(2)$	$4.6\pm4.1(2)$	$22.5\pm0.0(1)$	$14.2\pm0.0(1)$
Day 10	$10.8^{a}\pm0.8(2)$	$27.6\pm5.2(4)$	$42.0^{b}\pm0.0(1)$	$31.2\pm0.0(1)$
Day 15	$16.9\pm3.4(4)$	$21.8\pm2.3(2)$	$20.7\pm0.0(1)$	$32.2\pm2.1(2)$

[1]There were two populations of mice based on age. One group was less than 180 days of age (< 180 days), the other greater than 180 days of age (> 180 days).

[2]Mean ± SEM (n number). Means in the same row sharing different superscripts are significantly differet ($P < 0.05$).

DISCUSSION

In this study we compared the reproductive capacity of lethal yellow (Ay/a) and littermate black (a/a) females in order to define how Ay causes reproductive failures. Our principal assay was the serum progesterone analysis of nonpregnant and pregnant females. However, we also measured a number of other reproductive variables that provide valuable background information on the generation of Ay-induced infertility.

The analysis of reproductive parameters of Ay/a mice revealed that Ay/a mice have larger body masses than age-matched control littermates. Ay/a females weighed 11.7 grams more than controls. The Ay/a animals less than 180 days of age were significantly ($P < 0.01$) larger than the age-matched a/a controls, while Ay/a females greater than 180 days of age were numerically larger than age-matched controls (40.9 versus 25.0 grams, respectively). Thus, as Ay/a animals age, they accumulate more adiposity as was documented in the body percentage of fat and lean tissue represented by the ovarian fat pads and gastrocnemius muscle (Table 2). In both age groups of Ay/a and a/a littermates the Ay/a females had significantly ($P < 0.01$) larger mean percent body weight of the right and left ovarian fat pads and numerically the smallest mean percentage of body weights of right and left gastrocnemius. Yellow animals are producing significantly more adipose tissue than the control animals.

Ay/a ovaries weighed numerically more than those of a/a controls. However when ovarian weights were compared on the basis of

12 total follicles. Although not significantly ($P < 0.05$) different, these data may indicate an ovarian-uterine asynchrony on the part of the Ay/a animals as they age.

That Ay/a and a/a ovarian activities are similar is also supported by the results of the mechanically liberated ova from right ovaries. The total number of liberated ova was numerically similar between the two genotypes. Ay/a females actually had the highest mean total liberated ova (16.3 versus 15.0) indicating the ovary is functional (as judged by mechanically-liberated ova) in obese yellow mice greater than 180 days of age. However, total liberated ova is lower ($P < 0.05$) in the Ay/a females greater than 180 days compared to the Ay/a females less than 180 days. No such age effect was observed in a/a controls (Table 3).

Mechanically liberated ova were broken down into three subpopulations, naked ova, ova with granulosa cells, and liberated follicles with ova. There were very few numerical differences in mean numbers in each of these subpopulations between age-matched genotypes. With respect to follicles containing ova (Table 3) the majority were liberated in young a/a females. These observations support the hypothesis that the Ay/a ovary, even in obese females, is functional and does not possess one or more intrinsic defects. Based on reciprocal ovary grafts between Ay/a and a/a females, Granholm and Dickens (1986) also concluded that the Ay/a ovary is not intrinsically defective.

Histological comparisons of folliculogenesis in Ay/a and a/a

mice supports the hypothesis that genetically yellow ovaries are normal. In both genotypes, the number of preantral follicles, Graafian follicles, and corpora lutea were numerically similar between age-matched genotypes. The mean number of antral follicles present was identical in the two genotypes. The mean number of atretic follicles was also consistent within all four groups (Table 4).

Ay/a ovaries exhibited more stromal tissue than age-matched controls; 36% (5/14) of the Ay/a females exhibited more than 50% stroma of ovarian transverse sections. In contrast, 17% (2/12) of the a/a female ovaries were composed of more than 50% stroma. The slight increase in mean weight of the Ay/a ovary compared to the age-matched a/a ovary may be due to this increase in stroma.

The ovary of the Ay/a mouse appears to be a normal functioning organ. However, if the Ay/a ovary does not receive proper signals from the pituitary, it cannot function normally and may not synthesize and release control levels of ovarian steroids. The findings of Granholm and Brock (1981) and Granholm et al. (1986) relating to the decreased reproductive rates of Ay/a females over the age of 120 days, lends support to the suggestion that Ay/a ovaries do not produce control levels of ovarian steroids.

Upon comparing genotypes with respect to ovulation rates via the flushings of the reproductive tracts (Table 3), no statistical differences occurred between Ay/a and a/a females of the mean number of normal ova flushed from the reproductive tracts of the two genotypes. Interestingly, only 13.6% (3/22) of Ay/a females had

degenerated ova compared to 29.6 (8/27) a/a females. The fewer degenerative ova in the Ay/a tracts may be due to the decrease in ovulatory activity of the Ay/a females (Granholm et al., 1986).

Ay/a ovaries appear to be normal in their ability to produce ova yet show a decrease in ovulation (Granholm et al., 1986). This may be due to aberrant steroid activity in Ay/a females.

Analyses of uterine weights also support an Ay-induced aberrant ovarian steroid production. The preparation and maintenance of a receptive uterus and healthy uterine environment prior to and during implantation depends upon progesterone and estrogen in precise amounts at precise times (Beamer et al., 1983; Whittingham and Wood, 1983). Decreased uterine weights at Ay/a females would strongly suggest an Ay/a induced ovarian steroid imbalance.

Uteri of Ay/a females weighed less than those of the age-matched a/a control littermates. Older mice in both genotypes have numerically smaller uteri. The greatest difference was between uteri of the Ay/a females less than 180 days of age versus the Ay/a females greater than 180 days of age. This supports the notion that Ay induces a graded or gradual infertility rather than an abrupt cessation of reproduction.

To determine steroid levels in Ay/a females, serum progesterone concentrations were determined on all sacrificed Ay/a and a/a mice by means of RIA. Progesterone is the dominant steroid following ovulation. Prior to ovulation the outer layer of granulosa cells in the Graafian follicles starts to synthesize progesterone. Upon the

formation of the corpus luteum, the principle steroid produced is progesterone. If progesterone levels are normal in the Ay/a females, the Ay/a ovaries would be functionally normal with respect to progesterone synthesis and release. In contrast depressed progesterone levels could mean:

a) Ay/a pituitary glands are producing insufficient quantities of gonadotropins or they are producing normal levels of defective (biochemically altered) gonadotropins,

b) Control levels of normal gonadotropins are being produced and secreted, but their concentration at the ovary is low because they are being diluted due to metabolic changes in fat tissue. There simply is not sufficient gonadotropin activity within follicular ovarian cells to promote ova maturation and ovulation,

c) Control levels of normal gonadotropins are being produced, secreted and presented to ovarian target cells. However, Ay is effecting, in some way, the ability of these target cells to receive, internalize and respond to the normal signals, and/or

d) Combinations of a, b, and/or c.

The mean progesterone concentration (ng/ml) were higher in the total population of Ay/a females (11.8 ng/ml) versus the control females (7.7 ng/ml). Both genotype populations were small (37 yellow and 40 black animals). Although n numbers were low, an interesting trend developed which was consistent in most of the genotype comparisons of progesterone levels. Ay/a females less than 180 days of age had significantly higher ($P < 0.01$) progesterone levels (13.9

ng/ml) than age-matched a/a mice (6.2 ng/ml). The older Ay/a mice (greater than 180 days of age) had numerically higher progesterone levels (10.2 ng/ml) than the age-matched a/a females (9.9 ng/ml). Progesterone levels of Ay/a mice decreased as they aged whereas those of a/a mice increased. Elevated progesterone levels may be associated with the loss of fertility in the Ay/a females. Nelson et al. (1981) reported irregular estrous cycles (prolonged cycles) in aging rodents prior to cessation of estrus; such prolonged cycles may lead to elevated progesterone levels.

In normally cycling nonmated female mice, Graafian follicles produce progesterone prior to ovulation. Once ovulation occurs progesterone is the dominant hormone throughout the luteal phase which lasts from 2 to 3 days in mice. Following the luteal phase the corpus luteum involutes and becomes atretic. The luteal phase of follicular development occurs during metestrus and diestrus of the estrous cycle (Whittingham and Wood, 1983).

Nelson et al. (1981) using C57BL/6J female mice found that 75% of the mice exhibiting prolonged cycles had diestrus-type smears on Day 5 through Day 7. In normally cycling mice (i.e., those having a 4-5 day estrous cycle) estrogen levels are significantly greater on days 3 and 4 of the five-day cycle than plasma estrogen levels on days 3 and 4 of older mice exhibiting prolonged estrous cycles (i.e., 150 days and 280 days of age, respectively.

Prolonged estrous in aging mice are cycles caused by differing profiles of the midcycle elevation of plasma progesterone (Nelson et

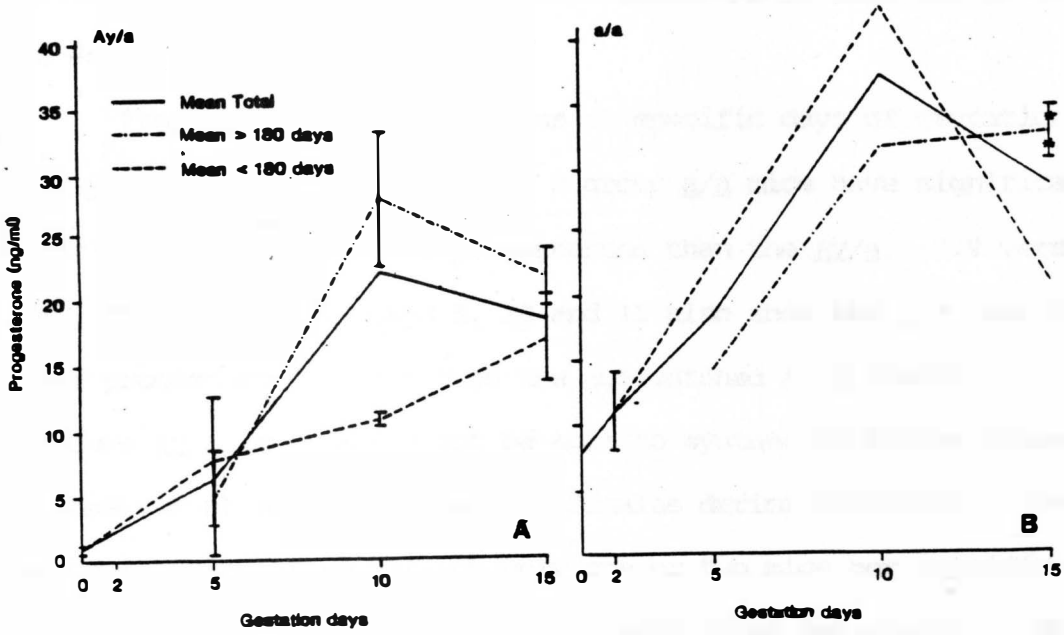
al., 1981). Midday levels represent basal as well as peak progesterone concentrations. In rats with prolonged cycles the midcycle increase of progesterone on day four is extended to day five. However it is not known if the elevated progesterone inhibits the action of estrogen (Nelson et al., 1981).

Supporting the hypothesis of prolonged cycles in Ay/a females, 72.2% (13/18) of nonpregnant Ay/a females were scored in metestrus and diestrus stages of the estrous cycle. In contrast, a/a mice tested for estrous stages via vaginal smears were distributed evenly throughout the four estrous stages. When estrous stages were compared to the progesterone levels of the age-matched genotypes, yellow mice had higher progesterone levels in all stages with the exception of the animals greater than 180 days. In these mice a/a and Ay/a females had serum progesterone levels of 17.8 and 8.6 ng/ml, respectively.

Upon comparing progesterone levels and mating status of the two genotypes similar results were found. Ay/a female progesterone levels were higher in six of eight mating statuses when compared to a/a females. Differences between progesterone levels of pregnant mice do not reflect the stage of pregnancy, since females within genotypes were grouped together. Also, elevated levels of progesterone for females that displayed vaginal plugs but were not pregnant, may be due to neuroendocrine stimuli they received during copulation. The stimulated cervix sends messages to the CNS and activates the release of gonadotropins and prolactin from the pituitary. These hormones maintain corpora lutea which then produce more progesterone. The

luteal phase in infertile matings will last from 11 to 12 days due to this luteotrophic effect of prolactin.

Upon comparing mean progesterone levels (ng/ml) of Ay/a and a/a (Figures 1A, 1B, respectively), with the results of Murr et al. (1974) (Figure 1C) on specific days of pregnancy our experimental animals showed similar characteristics. From day 0 to day 2 an increase in progesterone concentrations are found in all three graphs. Figure 1C shows a plateau from days 3-9. While the same plateau is not evident in our day 5 animals. In all three figures (Figures 1A-C) there is a slight increase in progesteron level from day 2 to day 5. Realizing that our data may be inconclusive due to the small sample, our first nonconformity with Figure 1C is found at day 10. Murr et al. (1974) reported a slight drop in progesterone level precisely at day 10 "... during the period when the pituitary becomes dispensable for the maintenance of pregnancy .... and the placenta is able to assume complete steroidogenic control". The mean progesterone level Murr et al. (1974) reported on day 10 was lower than that on day 5. Our day 10 levels of progesterone were higher in both Ay/a and a/a mice than their respective genotypes at day 5. It is interesting that we observed the highest concentration of progesterone in both genotypes in day 10. In contrast, Murr reported the output of progesterone during the latter part of gestation far exceeds that of the first half. This increase could be due to the influence of placenta luteotropins on luteal activity; between days 9 and 13 there is an increase in luteal growth (Murr et al., 1974). Both Murr et al.



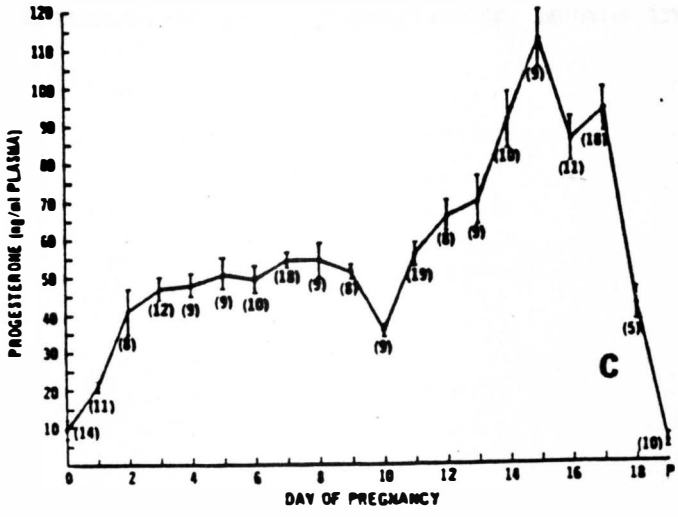


Figure 1. Progesterone concentrations, expressed as means and standard errors in serum throughout pregnancy. Mean for total mice populations represented by (_____), mean for mice populations greater than 180 days of age represented by (—.—), and mean for mice less than 180 days of age represented by (— —). Figure 1-A is the progesterone concentrations of the pregnant Ay/a females. Figure 1-B is the progesterone concentrations of the control a/a females while Figure 1-C is a reproduction from a paper by Murr et al. (1974) "Plasma Progesterone During Pregnancy in the Mouse".

(1974) and Pointis (1941) report peak progesterone levels on day 15. In both black and yellow genotypes progesterone concentrations declined from day 10 to day 15.

Progesterone concentrations at specific days of gestation are displayed in Table 7. In the day 0 group a/a mice have significantly higher ($P < 0.01$) levels of progesterone than the Ay/a, (7.9 versus 0.9 ng/ml, respectively). Days 5, 10 and 15 also show the a/a females with higher progesterone levels than the age-matched Ay/a females. Therefore Ay/a females may not be able to synthesize progesterone to the same extent as age-matched a/a females during gestation. However, these data which often reflect only one or two mice per specific gestation day may not reveal the true biological variations in the progesterone levels. Nonetheless, these data do show a general overall depression of serum progesterone levels in pregnant Ay/a mice.

SUMMARY

The following summary statements can be drawn from this study:

1) Of the numerous tests conducted on the reproductive potential of yellow mice, no differences were shown between yellow and control mice. Therefore, Ay appears to induce a graded or gradual infertility rather than an abrupt cessation of reproduction.
2) Based on studies of ovary size, visually scored follicles, mechanically liberated ova, and histological comparisons, Ay/a ovaries appear to be normal in their ability to produce ova; yet Ay/a ovaries show a decrease in ovulation. These observations suggest that Ay defects are extrinsic to the ovary; Ay/a ovaries may receive improper signals from the pituitary.
3) Data on progesterone assays suggest the following:
   a) Progesterone levels decreased in Ay/a females as they aged whereas serum progesterone levels increased in aging controls.
   b) Overall levels of progesterone were higher in Ay/a females than in age-matched controls.
   c) Progesterone levels of Ay/a and a/a mice measured on specific days of gestation seemed to be delayed or out of synchrony when compared to Murr's data.
   d) Overall, serum progesterone in pregnant Ay/a mice is depressed when compared to that of pregnant age-matched littermates.
4. Ay/a mice exhibited prolonged estrous cycles. A total of 72.2% of the nonpregnant Ay/a mice were judged in metestrous and diestrus in which progesterone is the dominant ovarian steroid.

5. Present results suggest the hypothesis that defects may exist which operate extrinsically to the ovary causing infertility in Ay/a females. The hypothalamus-pituitary axis is a likely site for this reproductive lesion. The increased adiposity of Ay/a mice may alter the feedback relationships between ovarian steroids and hypothalamic receptors resulting in a general depression of reproductive function.

LITERATURE CITED

Atkinson, W.B. and C.W. Hooker. 1945. The day-to-day level of estrogen and progestin throughout pregnancy and pseudopregnancy in the mouse. Anatomical Records 93:75-79.

Austin, C.R. and R.V. Short. 1984. Hormonal control of reproduction, 2nd ed., Cambridge University Press, Cambridge.

Bartke, A.J. andn G.W. Wolff. 1966. Influence of the Lethal Yellow (A^Y) Gene on Estrus Synchrony in Mice. Science 153:79-80.

Beamer, W.G., M.C. Wilson and E.H. Leiter. 1983. Endocrinology. In: the mouse in biomedical research, Vol. III, pp. 165-245. Eds. H.L. Foster, J.D. Small and J.C. Fox. Academic Press, New York.

Bray, G.A. and D.A. York. 1979. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiological Review 59:719-809.

Charlton, H.M., D.M.G. Halpin, Carol Iddon, R. Rosie, G. Levy, I.F.W. McDowell, A. Megson, J.F. Morriss, A. Bramwell, A. Speight, B.J. Ward, J. Broadhead, G. Davey-Smith and G. Fink. 1983. The effects of daily administration of single and multiple injections of GuRH and pituitary and gonadal function in the (hpg) mouse. Endocrinology 113:535-544.

Cizadlo, G.R., S.K. Hoffman and N.H. Granholm. 1975. Mating characteristics and embryonic losses in the yellow (A^Y) mouse. Proc. S.D. Acad. Sci. 54:157-161.

Dickens, G.A. and N.H. Granholm. 1986. Effects of ovary grafting on reproductive performance of lethal yellow mice (A^Y/a; Strain C57BL/6J). Proc. S. D. Acad. Sci. 65:ABS.

Flurkey, K., D. Gee, N.Y. Sinta, J.R. Wisner and C.E. Finch. 1982. Age effects on luteinizing hormone, progesterone and prolactin in proestrus and acyclic C57BL/6J mice. Biology of Reproduction 26:835-846.

Forbes, T.R. and C.W. Hooker. 1957. Plasma levels of progestin during pregnancy in the mouse. Endocrinology 61:281-285.

Granholm, N.H. and K.T. Brock. 1981. Effects of the lethal yellow gene (A^Y) on reproductive performance in strain C57BL/6J mice. Proc. S. D. Acad. Sci. 60:24-28.

Granholm, N.H. and G.A. Dickens. 1986. Effects of reciprocal ovary transplantation on reproductive performance of lethal yellow mice (Ay/a; strain C57BL/6J). J. Reprod. Fertil. 78:749-753.

Granholm, N.H., K.W. Jeppesen, and R.A. Japs. 1986. Progressive infertility in female lethal yellow mice (A^y/a; strain C57BL/6J). J. Reprod. Fertil. 76:279-287.

Hadley, M.E. 1984. Endocrinology. Prentice-Hall, Englewood Cliffs, New Jersey. 441.

Jeppesen, K.W. 1985. The relationship between obesity and fertility in the lethal yellow mouse (A^y/a; C57BL/6J). Thesis at SDSU.

Johnson, L.M. and R. Sidman. 1979. A reproductive endocrine profile in the diabetes (db) mutant mouse. Biol. Reprod. 20:552-559.

Johnson, M. and B. Everitt. 1984. Essential reproduction, 2nd ed., Blackwell Scientific Publications, London.

Rakihana, R., L.B. Ellis, S.A. Gerling, S.L. Blum and S. Kessler. 1974. Bruce effect competence in lethal yellow mice. J. Reprod. Fert. 40:483-486.

McCormack, J.T. and G.S. Greenwald. 1974. Progesterone and oestradiol-17B concentrations in the peripheral plasma during pregnancy in the mouse. Journal of Endocrinology 64:101-107.

Murr, S.M., G.H. Stubenfeldt, G.E. Bradford and I.I. Geschwind. 1974. Plasma progesterone during pregnancy in the mouse. Endocrinology 94:1209-1211.

Nelson, J.F., L.S. Felicio, H.H. Osterburg and C.B. Finch. 1981. Altered profiles of estradiol and progesterone associated with prolonged estrus cycles and persistent vaginal cornification in aging C57BL/6J mice. Biology of Reproduction 24:784-794.

Pointis, G., B. Rao, M.T. Latreille, T.M. Mignot and L. Cedard. 1941. Progesterone levels in the circulating blood of the ovarian and uterine veins during gestation in the mouse. Biology of Reproduction 24:8001-805.

Rafferty, Jr., K.A. 1970. Methods in experimental embryology of the mouse. Johns Hopkins Press, Baltimore.

Rugh, Robert. 1968. The mouse reproduction and development. Burgess Publishing Co., Minneapolis, MN

Swerdloff, R.S., M. Peterson, A. Vera, R.A. Batt, D. Heber and G.A. Bray. 1978. The hypothalamic-pituitary axis in genetically obese (ob/ob) mice; response to luteinizing hormone-releasing hormone. Endocrinology 103:542-547.

Virgo, B.B. and G.O. Bellwald. 1974. Serum progesterone levels in the pregnant and post partum laboratory mouse. Endocrinology 95:1486-1490.

Whitten, W.K. 1973. Genetic variation of olfactory function in reproduction. Journal of Reproduction and Fertility Suppl. 19:405-410.

Whitten, W.K., A.K. Champlin. 1978. Methods in Mammalian Reproduction. Ed: J.C. Daniel. Academic Press, New York, 403-417.

Whittingham, D.G. and M.J. Wood. 1983. Reproductive physiology. In the Mouse in Biomedical Research. Vol. III, pp 138-164. Eds. H.L. Foster, J.D. Small and J.C. Fox. Academic Press, New York.

Young, L.S., A. Detta, R.N. Clayton, A. Jones, and H.M. Charlton. 1985. Pituitary and Gonadal Function in Hypogonadotrophic Hypogonadal (HPG) Mice Bearing Hypothalamic Implants. J. Reprod. Fertil. 74(1):247.

Appendix IA. Raw data on reproductive parameters of lethal yellow mice.

Mouse -No.	Age (Days) at Sacrifice	Body wt (grams)	Tail length (mm)	Mating status[a]	Estrus stage[b]	Wt RO fat pad (mg)	Wt LO fat pad (mg)	Wt RT gastrocnemius (mg)
1	197-2[f]	36.4	88	1		34.4	40.0	109.6
3	199-2	39.2	88	3	1	40.2	47.1	39.1
5	268-2	39.4	89	3	4	51.4	42.3	107.6
7	269-2	30.3	85	3	3	11.2	16.5	70.9
9	269-2	51.3	87	3	4	59.4	57.9	32.2
11	164-1	47.3	87	3	3	331.8	34.7	106.2
13	203-2			3				
15	180-1	36.1	89	3	3	34.6	27.0	55.8
17[d]	167-1			4				
19[d]	167-1			4				
21[c]	268-2			3				
23	256-2	34.1	88	2		20.3	61.4	34.5
25	263-2	40.2	85	1		61.8	39.5	123.7
27	260-2	39.4	85	3	4	58.8	67.9	91.4
29[e]								
31	257-2	36.0	90	2		60.1	29.8	73.3
33	257-2	43.5	88	1		38.2	137.4	56.6
35	258-2	42.0	88	4		27.0	42.7	85.0
37	258-2	44.5	86	4		68.0	94.3	83.2
39	247-2	41.9	88	3	4	64.1	36.6	75.9
41	248-2	47.3	84	3	4	72.1	56.5	59.1
43	250-2	46.0	94	1		74.6	99.4	120.0
45	247-2	39.3	89	3	4	57.7	33.6	78.3
47	247-2	42.0	84	3	1	25.6	69.4	93.7
49	248-2	39.5	89	3	1	34.6	100.0	142.5
51	129-1	26.8	91	1		20.9	9.1	60.6
53	128-1	24.2	84	3	4	7.4	6.9	67.8
55	123-1	24.8	84	1		11.2	6.6	136.3
57	138-1	26.5	88	1		3.8	11.5	63.4
59	264-2	47.3	87	3	2	91.6	63.8	151.7

Appendix IA (continued).

Wt left gastrocnemius (mg)	Wt RO (mg)	Wt LO (mg)	Wt of uterus (mg)	Progesterone level (ng/ml)
100.0	11.0	---	107.2 -1[a]	4.2-5[g]
57.6	7.2	3.3	93.2-3[a]	1.3
107.6	4.5	4.5	43.1-3	2.2
42.1	3.5	2.3	47.1-3	0.9
79.7	3.5	7.7	44.0-3	1.2
101.7	5.2	4.0	54.8-3	23.3
76.8	1.1	3.7	49.7-3	6.0
93.6	3.2	2.8	67.1-2	0.5
92.6	4.5	5.2	536.6 -1	31.2-10
114.1	2.9	8.3	56.3-3	2.8
75.9	5.8	3.0	54.5-2	15.6
84.6	7.8	3.8	67.0 -1	4.6-0
95.5	4.9	7.3		34.3-15
105.0	4.4	2.5		30.0-15
77.3	3.8	1.3	56.0-3	1.3
61.4	3.3	2.8	41.1-3	1.1
94.3	3.1	3.7	79.8 -1	14.2-5
101.4	4.1	4.2	46.0-3	22.9
67.8	5.9	6.9	49.2-3	8.3
136.0	1.9	7.0	52.9-3	1.5
72.0	7.9	3.7	90.3 -1	0.5-5
66.8	4.3	2.8	51.7-3	5.4
128.1	6.2	4.0	135.9 -1	0.6
72.6	3.9	2.1	863.8 -1	20.4-10
163.1	6.1	3.3	48.5-3	2.1

Appendix 1A (continued).

Mouse -No.	Age (Days) at Sacrifice	Body wt (grams)	Tail length (mm)	Mating status[a]	Estrus stage[b]	Wt RO fat pad (mg)	Wt LO fat pad (mg)	Wt RT gastroc-nemius (mg)
61	261-2	42.9	81	4	4	24.6	27.4	99.2
63[c]	129-1			3				
65	127-1	26.8	88	1		28.6	4.1	95.6
67[e]	127-1			4				
69	125-1	29.4	89	1		16.5	8.3	132.1
71[c]	127-1			3				
73[c]	127-1			3				
75	129-1	30.7	86	3	4	36.8	20.2	62.0
77[c]	136-1			3				
79	127-1	21.3	87	2		2.8	8.6	71.3
81[c]	127-1			3				
83[c]	127-1			3				
85	118-1	23.6	85	1		7.6	5.8	68.6
87	126-1	30.4	88	1		6.7	13.7	90.6
89[c]	127-1			3				
91[c]	164-1			3				
93	153-1	37.1	84	1		24.7	35.9	73.7
95	157-1	41.2	88	1		54.9	43.9	54.9
97	154-1	30.5	84	2		26.1	16.1	72.1
99	160-1	32.1	90	3	1	46.6	25.6	76.2
101	126-1	36.0	91	4	4	81.1	17.1	109.6

Appendix IA (continued).

Wt left gastrocnemius (mg)	Wt RO (mg)	Wt LO (mg)	Wt of uterus (mg)	Progesterone level (ng/ml)
97.8	7.2	2.8		28.4-15[g]
95.8	4.9	4.1	729.6 -1[a]	19.7-10
138.0	11.4	8.4		24.1-15
83.3	2.5	4.2	85.4-3	0.8
46.5	4.1	2.3	112.4-2	0.3
71.5	1.1	4.7	105.6 -1	8.6-5
81.9	3.2	3.0		19.5-15
83.9	4.4	4.9	114.8 -1	1.3-0
84.0	7.2	1.0		28.3-10
74.4	4.6	3.6	76.7-2	26.6
73.1	3.6	6.1	71.9-3	1.2
80.9	10.1	4.0	404.3 -4	41.9-10

[a]Mating status 1 = Vaginal plug and pregnant; 2 = Vaginal plug but not pregnant; 3 = Non-plugged and non-pregnant; 4 = Non-plugged and pregnant.
[b]Estrous stage 1 = Proestrus; 2 = Estrus; 3 = Metestrus; 4 = Diestrus.
[c]Mice given to Dr. Granholm for other projects.
[d]Mice gave birth.
[e]Mice died during project.
[f]Age 1 = 120-180 days of age; 2 = Greater than (>) 180 days of age.
[g]Days of gestation -- 0, 2, 5, 10 or 15 days.

Appendix IB. Raw data on reproductive parameters of black mice.

Mouse -No.	Age (Days) at Sacrifice	Body wt (grams)	Tail length (mm)	Mating status[a]	Estrus stage[b]	Wt RO fat pad (mg)	Wt LO fat pad (mg)	Wt RT gastroc-nemius (mg)
2	209-2[f]	23.5	86	3	3	8.4	6.8	94.8
4	211-2	19.6	85	3	1	4.5	5.7	52.5
6	210-2	20.5	91	3	3	6.5	3.1	95.8
8	270-2	26.5	89	3	2	8.3	7.4	71.8
10	270-2	23.6	91	3	2	6.4	7.6	64.5
12	174-1	23.3		3	1	5.4	2.5	119.3
14	182-2	22.7	84	3	1	1.5	1.9	91.8
16	184-2	39.1	90	4	4	12.3	2.8	112.8
18	149-1	20.0	82	1		29.3	9.0	89.6
20	241-2	24.5	92	2		21.0	34.9	108.6
22	241-2	25.5	88	1		6.2	9.6	113.3
24	247-2	21.6	86	2		8.9	11.1	84.4
26	247-2	22.8	87	1		15.9	7.1	94.1
28	245-2	22.1	88	3	2	3.9	1.8	96.3
30[c]	263-2			3				
32	256-2	22.5	86	2		4.6	3.8	53.6
34[c]	263-2			3				
36[c]	263-2			3				
38[c]	250-2			3				
40	228-2	23.4	86	2		16.2	9.7	92.1
42	243-2	32.8	88	4	4	61.5	2.0	83.6
44[c]	250-2			3				
46	239-2	29.3	89	1		6.7	10.5	96.9
48	149-1	25.6	89	1		7.5	10.7	63.2
50	139-1	22.7	88	2		5.5	3.3	137.9
52	128-1	20.6	84	3	1	4.5	1.9	73.3
54	128-1	20.1	84	3	2	8.1	6.0	65.8
56	145-1	22.0	86	3	2	3.3	3.9	85.3
58	127-1	22.0	86	2		6.3	3.7	77.9
60	142-1	21.7	82	3	2	0.3	3.8	60.4

Appendix IB (continued).

Wt left gastrocnemius (mg)	Wt RO (mg)	Wt LO (mg)	Wt of uterus (mg)	Progesterone level (ng/ml)
102.3	8.2	6.1	64.4-3[a]	2.6
62.4	4.5	3.8	79.3-3	4.3
85.9	0.4	1.0	91.6-3	1.4
74.7	3.2	4.3	58.9-3	0.7
66.6	3.8	4.5	41.9-3	1.3
115.6	5.8	3.7	53.2-3	2.2
72.9	4.0	3.1	55.8-3	1.6
121.6	11.9	6.9		14.9
92.7	2.6	2.5	97.6 -1[a]	1.6-0g
118.2	4.2	2.4	64.5-22	29.1
96.3	7.0	3.4	177.1 -1	7.9-0
79.4	3.9	2.0	68.9-2	0.9
93.5	3.9	1.9	118.0 -1	22.3-5
93.2	2.5	2.3	50.7-3	2.8
77.9	8.1	2.5	68.8-2	1.0
110.2	3.3	4.7	46.4-2	5.4
98.1	3.2	2.9		20.7-15
89.7	5.2	3.0	826.4 -1	42.0-10
79.8	5.9	5.7	130.0 -1	2.9-5
119.9	6.2	2.6	85.8-2	1.6
69.2	2.9	2.9	48.5-3	1.8
65.5	2.9	2.9	64.9-3	0.6
89.3	2.5	4.0	135.3-3	0.8
67.3	3.7	2.3	54.1-2	0.8
95.5	3.9	2.1	51.5-3	0.8

Appendix 1B (continued).

Mouse -No.	Age (Days) at Sacrifice	Body wt (grams)	Tail length (mm)	Mating status[a]	Estrus stage[b]	Wt RO fat pad (mg)	Wt LO fat pad (mg)	Wt RT gastrocnemius (mg)
62	145-1[f]	22.1	88	3	4	5.6	6.4	122.3
64	143-1	23.9	88	2		5.7	4.3	106.9
66	145-1	30.8		1		6.2	9.2	84.7
68[c]	146-1			3				
70	134-1	29.8	91	4		12.5	8.2	97.4
72	145-1	31.8	79	1		11.0	1.6	81.6
74	128-1	21.7	84	1		4.1	3.5	76.8
76	129-1	22.2	85	1		6.7	4.4	46.4
78[c]	136-1			3				
80[c]	136-1			3				
82	136-1	28.5	86	1		8.9	9.5	114.0
84	132-1	21.8	84	2		18.6	6.8	63.8
86[c]	136-1			3				
88	131-1	26.2	86	1		11.2	4.8	59.3
90	134-1	33.2	87	1		11.9	22.2	112.1
92	130-1	25.4	85	1		2.3	4.4	56.7
94[c]	136-1			3				
96	149-1	23.7	80	1		6.2	13.3	78.4
98	153-1	26.4	78	1		7.4	5.4	51.1
100	147-1	24.1	89	1		3.9	3.9	76.3

Appendix IB (continued).

Wt left gastrocnemius (mg)	Wt RO (mg)	Wt LO (mg)	Wt of uterus (mg)	Progesterone level (ng/ml)
119.6	7.2	4.0	65.1-3[a]	2.4
71.7	5.2	3.4	42.9-2	0.5
87.1	4.3	5.9		18.3-15[g]
117.6	5.9	4.6		4.6
119.8	7.5	2.5		12.1-15
61.4	3.7	2.6	50.9 -1	7.3-2
66.7	1.9	2.8	54.2 -1	14.9-2
100.8	3.4	5.5		11.4-15
69.6	3.8	1.9	49.3-2	2.2
60.0	3.2	4.5	811.8 -1	10.0-10
112.4	12.6	6.5		25.8-15
106.2	2.3	4.7	845.2 -1	11.5-10
92.5	4.6	2.9	137.2 -1	0.7
78.5	6.4	8.4	139.0 -1	12.8-5
71.3	4.1	2.6	157.0 -1	0.6-0

[a]Mating status 1 = Plugged and pregnant; 2 = False plugged; 3 = Non-plugged and non-pregnant; 4 = Non-plugged and pregnant.
[b]Estrous stage 1 = Proestrus; 2 = Estrus; 3 = Metestrus; 4 = Diestrus.
[c]Mice given to Dr. Granholm for other projects.
[f]Age 1 = 120-180 days of age; 2 = Greater than (>) 180 days of age.
[g]Days of gestation -- 0, 2, 5, 10 or 15 days.

Appendix IIA. Raw data on further reproductive parameters of lethal yellow mice.

ID No.	Flushed normal ova	Flushed degenerated ova	Embryos in early cleavage	Embryos in blastocyte stage	Gestation days	No. of deciduae	Mean wt of decid (mg)	Visual score or RQ[a]
1					6	10	6.2	3[a]
3	7							3
5	1							3
7	7							3
9	0							3
11	0							3
13[c]								
15	9							3
17[c]								3
19[c]								
21[c]								
23	0							3
25					10	10	3.8	3
27	0							3
29[d]								
31	0							3
33		3	3		1			3
35					15	9		3
37					15	9		3
39	0							3
41	0							3
43				2	5			3
45	0							3
47	0	6						3
49	7							3
51				4	5			3
53	0							3
55	9							3
57					10	9	52.1	3
59		1						3

Appendix IIA (continued).

Naked ova[b]	Liberated Ova with granular cells	Liberated follicles with ova	Total liberated ova	Preantral follicles	Small antral follicles	Graffian follicles	Corpora lutea	Artresic follicles	Stroma characters[e]
1	10	1	12						
1	6	6	13	14.0	3.3	3.0	5.0	3.0	2.0
3	12	2	17	5.0	1.7	3.7	2.7	3.7	3.0
5	6	1	12	8.3	3.0	3.7	4.0	1.7	1.0
2	13	3	18						
1	7	4	12	5.3	1.7	3.3	3.0	2.7	3.0
1	0	3	4						
2	4	2	8						
3	5	4	12						
2	7	5	14						
3	8	3	14						
4	7	3	14						
0	7	5	12	8.7	1.7	3.0	4.0	5.0	1.0
5	3	2	10						
6	2	6	14						
5	12	4	21						
5	5	14	24						
7	10	5	22						
3	3	8	14						
7	10	4	21	8.0	1.3	3.7	4.3	4.3	1.0
1	5	9	15	11.0	4.3	6.3	4.7	3.7	1.0
2	8	4	14	8.3	3.7	1.7	4.7	6.3	1.0
7	7	11	28						
4	7	3	14						

Appendix IIA (continued).

ID No.	Flushed normal ova	Flushed degenerated ova	Embryos in early cleavage	Embryos in blastocyte stage	Gestation days	No. of deciduae	Mean wt of decid (mg)	Visual score or RO[a]
61					14	5		3[a]
63								
65					10	7	47.5	3
67[c]					14	6		
69					15	8		3
71								
73[c]								
75		2						3
77[c]								
79	0							3
81[c]								
83[c]								
85					5	7	2.2	3
87					15	9		3
89[c]								
91[c]								
93	6				0			3
95					10	8	95.6	3
97	0							3
99	9							3
101					10	6	25.5	3

Appendix IIA (continued).

Naked ova[b]	Liberated Ova with granular cells	Liberated follicles with ova	Total liberated ova	Preantral follicles	Small antral follicles	Graffian follicles	Corpora lutea	Artresic follicles	Stroma characters
4	9	0	13						
7	4	7	18						
3	7	2	12						
3	4	10	17						
5	3	8	16						
4	11	6	21	8	4.7	4	3.3	3.3	1
2	9	7	18	5	3.3	1.7	3.3	2.7	1
3	15	8	26	8.4	2.3	2.0	3.3	8.6	
5	7	12	24	5.7	1.3	2.7	4.3	2.3	1
3	10	7	20						
1	9	5	15	4	3.7	3.7	1.3	2.3	3
0	4	1	5	4	0.7	1.3	3.0	1.7	3

[a]Visual score of the follicular development of right ovary. 1 = 1 to 5 follicles; 2 = 6 to 12 follicles; 3 = 13 plus follicles.
[b]Naked ova possess few, if any, granulosa cells.
[c]Mice given to Dr. Granholm for other projects.
[d]Mice died during project.
[e]Stroma character determined by much area of ovarian cross section is stromal tissue. 1 = <50%; 2 = 50%; 3 = >50%.

Appendix IIB. Raw data on further reproductive parameters of black mice.

ID No.	Flushed normal ova	Flushed degenerated ova	Embryos in early cleavage	Embryos in blastocyte stage	Gestation days	No. of deciduae	Mean wt of decid (mg)	Visual score or RO[a]
2	6							3[a]
4	9							3
6	2							2
8	9							3
10	7							3
12	8	1						3
14	6							2
16					18	8		3
18	6	5			0			3
20	0							3
22	8	3			0			2
24	2	1						2
26					5	8	3.6	3
28	7							3
30[d]								
32	0	1						3
34[d]								
36[d]								
38[d]								
40	7							3
42					15	7	4.3	3
44[d]								
46					10	10	43.4	3
48					5	10	3.9	3
50	5							3
52	0	9						3
54	0							3
56	0	1						3
58	0							3
60	5							3

Appendix IIB (continued).

Naked ova[b]	Liberated Ova with granular cells	Liberated follicles with ova	Total liberated ova	Preantral follicles	Small antral follicles	Graffian follicles	Corpora lutea	Artresic follicles	Stroma characters[c]
1	8	4	13	7.0	2.7	3.0	5.3	5.2	3.0
3	5	6	14	7.3	4.3	4.0	5.0	2.0	3.0
0	7	2	9	2.0	0	0.3	2.5	0.3	1.0
3	7	3	13	7.0	2.3	5.3	3.0	2.7	1.0
8	19	2	29						
3	6	9	18						
0	21	6	27						
4	1	2	7						
4	4	3	11						
3	11	3	17						
1	3	1	5	7.0	1.7	3.0	1.3	2.3	3.0
0	2	2	4						
3	3	4	10	7.7	1.7	3.0	5.0i	2.7	1.0
4	4	2	10						
3	4	7	14						
2	6	1	9						
6	6	6	18	2.3	2.0	1.3	3.0	1.7	1.0
6	3	6	15	8.0	2.1	3.0	3.0	1.3	1.0
8	14	5	27						
5	7	3	15						
7	12	6	25	11.0	1.3	3.0	3.0	5.3	1.0
2	7	9	18						
1	1	2	4						
4	9	7	20						
6	9	3	18	4.7	2.7	0.7	2.0	5.3	1.0

Appendix IIB (continued).

ID No.	Flushed normal ova	Flushed degenerated ova	Embryos in early cleavage	Embryos in blastocyte stage	Gestation days	No. of deciduae	Mean wt of decid (mg)	Visual score or RO[a]
62	7							3[a]
64	6							3
66					15	9		3
68[d]								
70					12	9		3
72					15	8	3	
74	4		8		2			3
76	1		7		2			3
78[d]								
80[d]								
82					15	5		3
84	2							3
86[d]								
88					10	9	52.9	3
90					15	9		3
92					10	9	53.8	3
94[d]								
96	9				0			3
98					5	7	7.3	3
100	10				0			3

Appendix IIB (continued).

Naked ova[b]	Liberated Ova with granular cells	Liberated follicles with ova	Total liberated ova	Preantral follicles	Small antral follicles	Graffian follicles	Corpora lutea	Artresic follicles	Stroma characters[c]
6	2	5	13						
6	14	14	34						
0	3	5	8						
6	4	4	14						
4	1	7	12						
7	8	8	23	4.7	4.7	2.3	3.0i	1.7	1.0
4	8	4	16	7.0	6.0	3.0	6.0	5.0	1.0
6	8	5	19						
6	2	4	12						
4	6	2	12						
4	0	0	4	4.7	2.0	2.3	2.7	3.7	1.0
3	8	5	16	1.3	1.3	0.3	5.0	4.7	1.0
3	9	11	23						
2	13	9	24	4.0	3.3	4.0	4.3	5.0	1.0
0	3	2	5	6.3	2.0	2.3	1.0	6.0	3.0

[a]Visual score of the follicular development of right ovary. 1 = 1 to 5 follicles; 2 = 6 to 12 follicles; 3 = 13 plus follicles.
[b]Naked ova possess few, if any, granulosa cells.
[c]Stroma character determined by much area of ovarian cross section is stromal tissue. 1 = <50%; 2 = 50%; 3 = >50%.
[d]Mice given to Dr. Granholm for other projects.